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Case Report

Immunohistochemistry characterization of a bronchioloalveolar carcinoma mixed subtype in cat

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Abstract

A 12 year-old mixed breed female cat was present with signs of dyspnea, cyanosis and drooling, followed by sudden death. At necropsy we observed nodules measuring 0.5 x 0.5 to 2.0 x 2.0 cm in the pulmonary parenchyma showed multifocal atelectasis associated with discrete emphysema. The histopathological examination of lung masses revealed neoplastic epithelial cells compatible with bronchioloalveolar carcinoma (BAC). The immunohistochemical analysis of tumor cells showed positive labeling for pan-Cytokeratin, CK 7, CK 20 and TTF-1. According to macroscopic features, as well as histological and immunohistochemical findings, this tumor was diagnostic as BAC mixed subtype.

Key words: feline, lung, immunohistochemistry, neoplasia.

Introduction

Lung cancer is the leading cause of cancer-related death worldwide and the second most common cancer in humans (3, 15). In contrast, is an infrequent found in domestic animals (2, 11). Spontaneous lung tumors are more frequently in dogs, cats and sheep (6, 8). A betaretrovirus is responsible for pulmonary adenocarcinoma in sheep (10), while, in the dogs and cats this neoplasia was not related to any infectious etiology (5, 6, 8). In dogs and cats, lung tumors occur as a sporadic geriatric disease and they have variety and clinical signs often similar to many other respiratory diseases, such as anorexia, weight loss, wheezing, lethargy, dyspnea, tachypnea, ataxia, hemoptysis, and coughing (6, 8, 9).

Various approaches to classify lung cancer in both humans and animals have used site of origin, histological pattern, or a combination of both (8, 9, 14). According to these studies, the main tumors types observed in dogs and cats are acinar adenocarcinoma, bronchioloalveolar carcinoma, adenosquamous and squamous cell tumors (3, 8, 9, 14). In the cat, the predominant pattern is adenocarcinoma, with less frequent adenosquamous or squamous and bronchioloalveolar subtypes (2, 4, 5, 14).

The aim of this study was to report the gross, histological and immunohistochemical features of a bronchioloalveolar carcinoma (BAC) mixed subtype in a female cat.

Case report

A 12-years-old, female, neutered, mixed-breed domestic cat no clinical or neoplastic history was presented to the Veterinary Pathology Service at the Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista (UNESP), Botucatu, Brazil. It presented clinical signals of dyspnea, cyanosis and drooling, followed by sudden death. Grossly, the lung showed multifocal atelectasis associated with discrete emphysema in all lobes. Also, in the lung parenchyma there were white nodules

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with smooth surface, firm consistency and multifocal distribution, measuring from 0.5 x 0.5 to 2.0 x 2.0 cm and severe impairment all lobes. The other organs did not have any significant gross lesions.

In the histopathological analysis of the nodules we observed two cellular types. The first one was composed by neoplastic epithelial cells inside the alveoli (some areas had a papillary growth) supported by delicate fibrovascular stroma. The cells were cuboidal, of medium size and distinct borders; their cytoplasm was granular and eosinophilic color. These neoplastic cells exhibited eccentric nuclei, elongated in shape, with finely stippled chromatin and prominent nucleoli (Fig. 1A, 1B). In this pattern was not observed mucin secretion by the neoplastic cells (Periodic acid-Schiff (PAS) stain was negative).

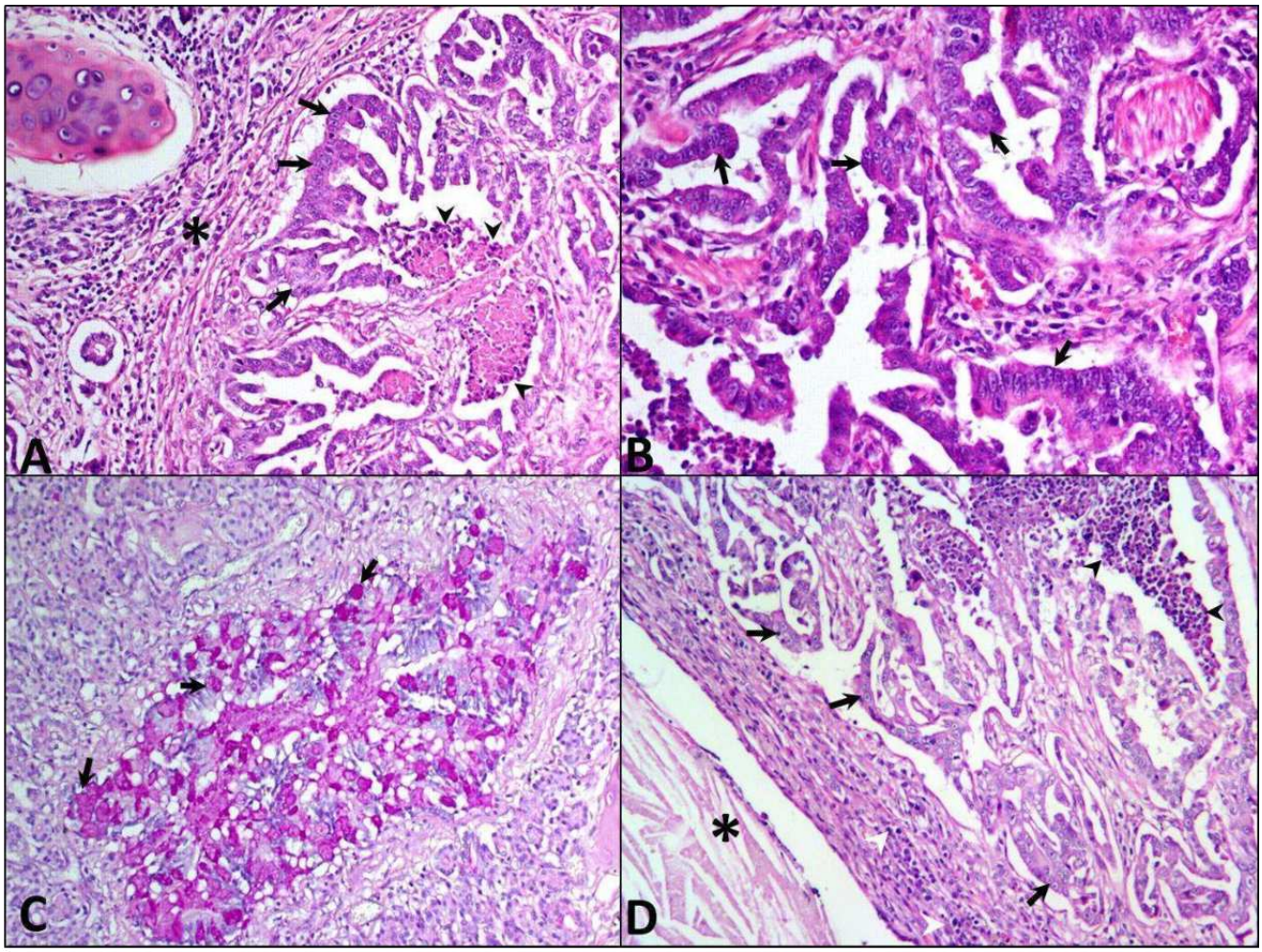


Figure 1. Bronchioloalveolar Carcinoma (BAC) mixed subtype, lung, feline. (A) The neoplastic cells of alveolar or papillary growth (arrows) were supported by a delicate fibrovascular stroma (asterisk), also is observed necrosis intraluminal (arrowheads), HE, (20x). (B) More detail of neoplastic cells. Granular cytoplasm, the cells also exhibited eccentric nuclei and prominent nucleoli (arrows), HE, (40x). (C) Areas of mucinous subtype tumor (arrows), circled by neoplastic cell of alveolar growth (asterisk), PAS, (20x). (D) The neoplastic cells (arrows), cholesterol clefts (asterisk), and inflammatory process composed by lymphocytes (white arrowheads) and granulocytic cells (black arrowheads) in the BAC, Hematoxylin and eosin (20x).

The second subtype was characterized by neoplastic epithelial cells of alveolar pattern supported by a discrete amount of fibrovascular stroma. The cells were columnar, of medium size and indistinct borders; their cytoplasm was abundant, frequently vacuolated and eosinophilic. Also was observed eccentric nuclei, round to oval with finely stippled chromatin. The neoplastic cells showed mucin secretion using PAS stain (Fig. 1C). Additionally, moderate comedonecrosis, atelectasis areas, emphysema, cholesterol clefts and inflammatory infiltrate composed mainly by lymphocytes were observed associated with the nodules (Fig. 1D). The regional lymph nodes did not present tumoral invasion.

Immunohistochemistry was performed using a Peroxidase system. Four slides were dewaxed in xylol and rehydrated in graded. For antigen retrieval the slides were incubated with citrate buffer (pH 6.0) in water bath for 30 minutes at 90°C or incubated in 0.1% pepsin in 0.01 N HCl (pH 2.0) for 15 minutes at room temperature, according to the primary antibody used (Table 1). The sections were treated with freshly prepared 3% hydrogen peroxide in methanol for 20 minutes, to inhibit endogenous peroxidase activity and then washed in Tris-bufferd saline.

The primary antibodies used were pan-Cytokeratin (AE1/AE3), Cytokeratin 7 (CK7), Cytokeratin 20 (CK20), Thyroid Transcription Factor 1 (TTF-1), the dilution and incubation for each antibody is present in Table 1. A polymer system (Envision, Dako, Carpinteria, CA, USA) was applied as the secondary antibody. After each step of the process, the slides rinsed with Tris-buffered saline. The slides were developed with DAB (Dako, Carpinteria, CA, USA) for 5 minutes and counterstained with Harris hematoxylin.

Immunohistochemistry was evaluated based on the distribution of positively stained tumor cells and by using the following scores: 1 (<25% positive cells), 2 (26% to 50% positive), 3 (51% to 75% positive), and 4 (>76% positive). Staining intensity in neoplastic cells was also evaluated as follows: 1 = weak staining, 2 = moderate staining and 3 = strong staining. The results of immunostained normal pulmonary parenchyma and tumor cells are described in the Table 2 and Table 3, respectively.

Positive staining was observed in neoplastic epithelial cells for all CKs (Fig. 2A, 2B, 2C). TTF-1 was positive in non mucin-secreting neoplastic cells (Fig. 2D). In according with these results the neoplasia was classified as BAC mixed subtype.

Table 1. Immunohistochemistry protocol and primary antibodies used.

Primary antibody	**Manufacturer**	**Dilution**	**Antigen-retrieval method**	**Incubation period**	**Secondary antibody**
Pan CK	Invitrogen	1:100	Citrate pH 6.0/WB	2 h/27 °C	Envision
CK 7	Dako	1:200	Pepsin pH 2.0	2 h/27 °C	Envision
CK 20	Biocare medical	1:200	Pepsin pH 2.0	2 h/27 °C	Envision
TTF-1	Dako	1:100	Citrate pH 6.0/WB	18 h/ 4 °C	Envision

Table 2. Localization of immunostaining in the normal parenchyma pulmonary.

	Cellular type								
Primary antibody	**Columnar ciliated cells**	**Columnar no ciliated cells**	**Glandular cells**	**Globet cells**	**Clara cells**	**Alveolar type I cells**	**Alveolar type II cells**	**Stroma**	**Alveolar MP**
	D+I	D+I	D+I	D+I	D+I	D+I	D+I	D+I	D+I
Pan CK	4+3	4+3	4+3	4+3	4+3	4+3	4+3	0	0
CK 7	4+3	4+3	4+3	4+2	4+2	4+3	4+3	0	0
CK 20	3+2	2+2	4+3	3+2	3+2	0	0	0	0
TTF-1	3+3	3+2	2+1	2+1	2+1	3+3	3+2	0	3+3

D+I, Distribution + Intensity of immunostaining. MP=Macrophages.

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Table 3. Localization of Immunostaining in the feline bronchioloalveolar mixed subtype (BAC).

	Cellular type			
Primary antibody	**Columnar ciliated cells** D+I	**Columnar no ciliated cells** D+I	**Globet cells** D+I	**Stroma** D+I
Pan CK	4+3	4+3	4+3	0
CK 7	4+3	4+3	4+2	0
CK 20	3+2	3+2	3+2	0
TTF-1	3+2	3+2	0	0

D+I, Distribution + Intensity of immunostaining.

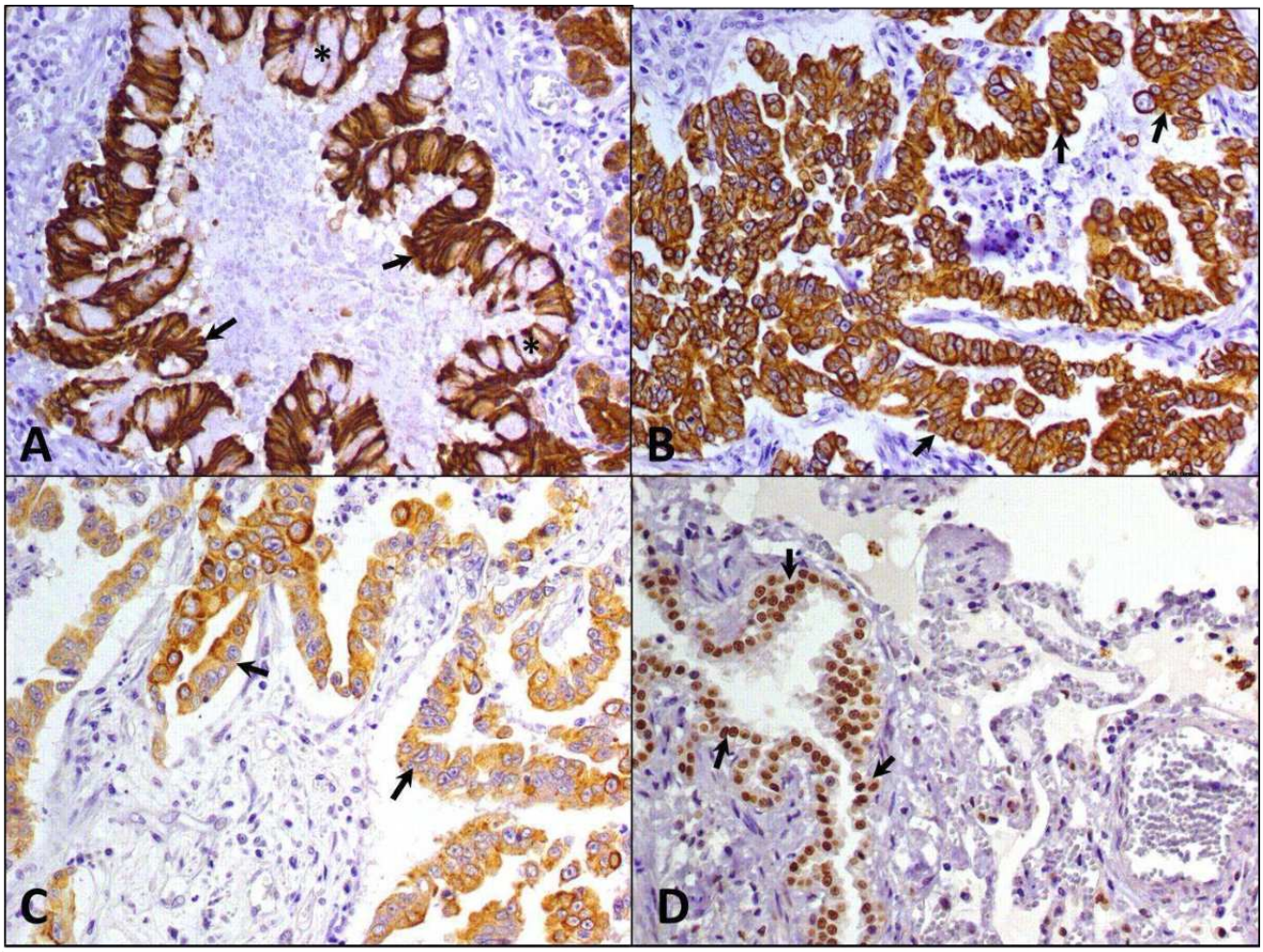


Figure 2. Immunohistochemical analysis in Bronchioloalveolar Carcinoma (BAC) mixed subtype, lung, feline. (A) Positive staining for pan cytokeratin antibody in the columnar ciliated cells (arrows) Globet cells (asterisk) and glandular cells (arrowheads), BAC, mucinous portion (40x). (B) Positive staining for Cytokeratin 7 antibody in the columnar ciliated cells (arrows), BAC, non-mucinous portion (40x). (C) Positive staining for Cytokeratin 20 antibody in the columnar ciliated cells (arrows), BAC, non-mucinous portion (40x). (D) Positive staining for Thyroid Transcription Factor 1 (TTF-1) antibody in non-mucinous neoplastic cells (arrows) (40x).

Discussion

Primary lung neoplasms are seen with more frequently in dogs and cats when compared with other domestic animals (1). In cats, the incidence of lung carcinoma has increased in the last years (2, 5, 14). This finding may be explained by longer lifespans and an increase in the number of animal necropsies being performed and reported (2, 14). This population is composed by older domestic cats, short hair, and with a mean age of 12 years.

Grossly, all subtypes of human BAC may appear as a solitary nodule, multiple nodules, or diffuse consolidation (15). In cat, BAC may occur as a diffuse consolidation (14) however, we observed multiples nodules in this animal. In the WHO classification, there are 3 subtypes of BAC: non-mucinous, mucinous and mixed (12). The BAC mixed subtype is composed of Clara cell, type 2 pneumocytes differentiation and tall columnar cells with bland, basally located nuclei and abundant apical cytoplasmic mucin (14, 15), theses cellular types also were observed in our case of feline BAC. Additionally, we used PAS stain to identify mucin-secreting neoplastic cells.

The lung is one of the main sites of metastases and, it is often difficult to distinguish primary pulmonary tumors from metastasis (7, 11). In our case, the absence of lesions in other organs corroborates that the neoplastic lesion is primary of the lung. Additionally, immunohistochemistry has been used to elucidate the histogenesis and help with the diagnosis of lung tumors in veterinary medicine. CK's and TTF-1 frequently serve as diagnostic markers for lung tumors (14).

In the respiratory system of dogs and cats, CK7 is positive in suprabasal ciliated columnar epithelium, in epithelium of respiratory bronchioles, in alveolar type 2 cells, and strongly in the mucosal glands (4). In normal canine lung, there are no CK20 positive cells, but in the cat, glandular cells and cylindrical ciliated epithelium have a strong reaction, and some positive cells can stain in the bronchiolar epithelium (4, 14), this expression pattern of CK7 and CK20 also was observed in the study.

In humans, most BACs are CK7 positive and CK20 negative, a pattern of immunoreactivity similar to that defined for pulmonary adenocarcinomas in general (7, 13, 15). When evaluated by subtype, CK7 is noted in neoplastic cells with or without mucinous secreting. In contrast, CK20 expression is absent in the non-mucinous BACs (7, 13). However, a $CK7^+/CK20^+$ staining can be seen in some mucinous and mixed bronchioloalveolar carcinomas in humans. The mechanism for aberrant expression of CK20 in humans BAC is unclear. Possible explanations include intestinal differentiation, oncofetal activation, or simply by an aberrant expression related to oncogenesis (13). In according to Lau et al. and Rajshri et al. (7, 13), our study also showed CK 7 and 20 protein expression with similar distribution (3 or 4) and intensity (2 or 3) in mucin secreting and non mucin secreting neoplastic cells in the bronchiolar epithelium (Table 3).

The differential diagnosis for $CK7^+/CK20^+$ adenocarcinomas also includes neoplasms with origins in the pancreas, ampulla, bladder, and ovary (3). To demonstrate the pulmonary origin of this tumor, we analyzed TTF-1 antibody by immunohistochemistry. In this report, TTF-1 expression was observed in areas with non mucin-secreting neoplastic cells, in accordance with results showed in humans BAC, which were positive TTF-1 for all BAC non-mucinous type (7). Also, alveolar macrophages were stained with TTF-1 but this positivity is considered unspecific. TTF-1 is a protein originally identified in follicular cells of the thyroid, but was also found in respiratory cells and areas of the developing brain (7). TTF-1 has been described as a highly specific marker in lung adenocarcinomas in human and dog (7, 11). D'Costa et al., (2) showed a labeling specificity of 66.7% in feline lung tumors and it was the highest percentage when compared with other antibodies Surfactant A (SP-A) (50%), Epidermal growth factor receptor (EGFR) (20.8%) and p53 (25%), suggesting the importance of these protein as marker to support the diagnosis of pulmonary tumors in the cat.

In this case, histochemical (PAS) and immunohistochemistry (CK7, CK20 and TTF-1) staining were useful for the diagnostic of pulmonary tumor and as well as their classification.

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